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Magnetic microbead sample handling integrated with optomagnetic nanobead detection

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Microfluidics provides different possibilities for high throughput nucleic acid tests to be performed in miniaturized "lab-on-a-chip" devices.[1] Magnetic microbeads (MMBs) coated with streptavidin are a standard substrate to handle biotinylated DNA targets, which allow for on-chip transportation of DNA anchored to the MMBs between microfluidic chambers by use of an external magnet. In this work, a DNA target was captured on a MMB, amplified by rolling circle amplification (RCA) using phi29 polymerase to form a long single stranded concatemer (the rolling circle product, RCP), which was subsequently detected via depletion of capture oligo-functionalized magnetic nanoparticles (MNPs) in a detection chamber as they bind to the RCP.[2] Here, we present the use of 1 µm MyOne magnetic beads (ThermoFisher) for magnetic transportation of DNA in a polymethylmetacrylate (PMMA) chip and investigate protocols with different polymerase concentrations during the RCA as well as the effect of adding EDTA to the detection chamber. We label the conditions P1, P2, P4, and P2 EDTA corresponding to the relative polymerase concentrations and as to whether EDTA is added.

The chip consists of three connected chambers of different sizes that contain the liquids for the reactions described below (Fig. 1a). The chip was fabricated using a CO_2 laser cutting and engraving process. In chamber I, circularized templates linked to the MMBs are magnetically separated from non-circularized probes by the use of an external magnet. In chamber II, isothermal RCA takes place for 45 min to produce large RCPs from the circularized probes. Then, the MMB-anchored RCPs are transported to chamber III that contains MNPs and the depletion of free MNPs was measured real-time for 1 hour using optomagnetic measurements. Fig. 1b shows the relative signal from free MNPs for conditions P1, P2, P4 and P2 EDTA as function of DNA target concentration (including no target controls, NTCs). The results showed capture of more MNPs for higher polymerase concentration indicating that more or longer RCPs were formed. As a compromise between signal and cost (the phi29 polymerase is expensive) we selected condition P2 for further studies. We further investigated impact of EDTA, which binds and dissolves magnesium salts formed during the RCA. Previous studies have shown the sponge-like RCPs to open and become more accessible for binding in the presence of 50 mM EDTA.[3] Our experimental results in Fig. 1b show that addition of EDTA results in a higher depletion of single MNPs, i.e., that the RCPs bind more MNPs, which is consistent with this. We therefore found that the P2 EDTA condition was optimum for our work.

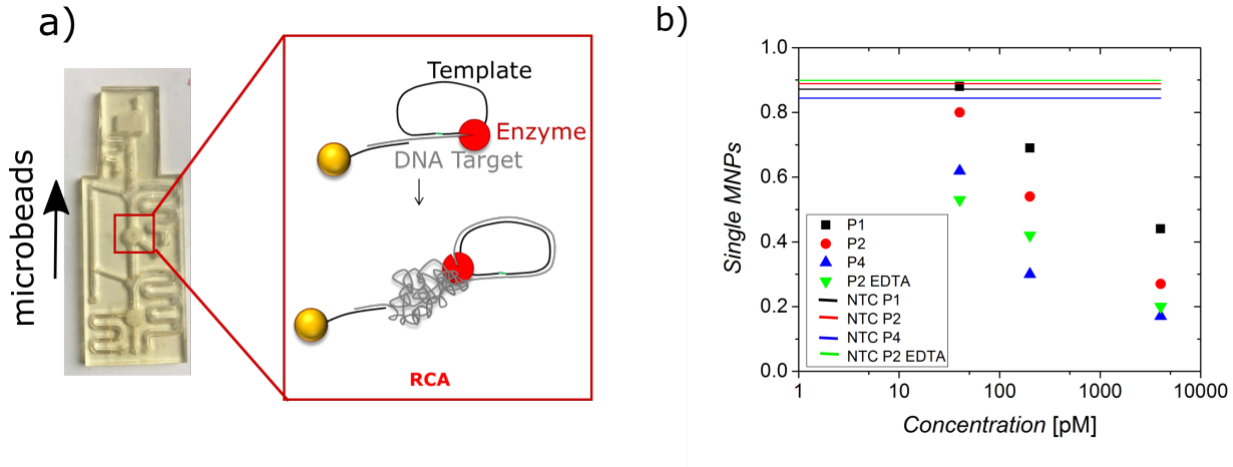

Figure 1 (a) Picture of PMMA chip. The arrow shows the direction of transport of the microbeads from chamber I to III**.** A schematic of the RCA process taking place in chamber II is shown. (b) Depletion of free MNPs obtained for the indicated conditions and DNA concentrations (including no template controls.

1. Lafleur JP, Jönsson A, Senkbeil S, Kutter JP. Recent advances in lab-on-a-chip for biosensing applications. Biosens Bioelectron. 2016;76:213–33.
2. Fock J, Jonasson C, Johansson C, Hansen MF. Characterization of fine particles using optomagnetic measurements. Phys Chem Chem Phys 2017;19(13):8802–14.
3. Roh YH, Lee JB, Shopsowitz KE, Dreaden EC, Morton SW, Poon Z, et al. Layer-by-layer assembled antisense DNA microsponge particles for efficient delivery of cancer therapeutics. ACS Nano. 2014;8(10):9767–80.